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Request for grant of a Patent

Form 1/77

Patents Act 1977

❶ Title of invention

1 Please give the title of the invention: DIAGNOSIS AND PREVENTION OF SPONGIFORM DISEASES

❷ Applicant's details

☑ **First or only applicant**

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Country *(and State of incorporation, if appropriate)*: UK

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Continuation sheets for this Patents Form 1/77			
Claim(s)		Description	8
Abstract	3	Drawing(s)	2

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HYPOTHESIS:

IS BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) AN AUTOIMMUNE DISEASE ?

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Summary:

Bovine spongiform encephalopathy (BSE) could be an autoimmune disease produced following exposure of cattle to feedstuffs containing bacteria showing molecular mimicry between bacterial components and bovine nervous tissue.

Analysis of molecular sequence databases (Genbank and SwissProt) shows that 3 bacteria (Acetinobacter calcoaceticus,Ruminococcus albus and Agrobacter tumefaciens) share sequences with the encephalitogenic peptide of bovine myelin, whilst 2 molecules in Escherichia coli show molecular mimicry with host encoded "prion" protein. Immune responses against these bacteria at both T and B cell levels, may cause neurological tissue injury resembling BSE. The role of these bacteria in BSE, if any, merits further investigation.

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INTRODUCTION

The relative increase in the late 1980's of bovine spongiform encephalopathy (BSE) in cattle in the United Kingdom has evoked some public interest. It appeared that this increase occurred after feeding cattle with ovine/bovine material, although since the practice has been discontinued, the number of BSE cases has steadily declined.(1)

Several theories have been proposed to explain this phenomenon, the most compelling being the "prion hypothesis".(2)

The "prion hypothesis" postulates that there is an infectious particle of a virus/prion nature which is transmitted to sheep (scrapie) and cows (BSE) and maybe even humans (Creutzfeldt-Jakob disease)(CJD). However there are several difficulties with this hypothesis.

(1) There is no structural evidence for the presence of the particle : There are no electron microscopy pictures of such an agent, there is no immunological evidence of infection and there are no microbiological methods available to grow such a virus/prion agent.(3)

(2) The "prion sequence" is actually encoded by the host (4) and is therefore a "self-protein" and probably not part of an external infectious agent.

(3) The human "prion sequence" which accumulates in brain lesions, KTNMKHMAGAAAAGAVVGGLG, consists mostly of aliphatic amino acids which readily polymerize into amyloid like fibrils (5). This could explain why these "self-proteins" are relatively resistant to hydrolysis by macrophage enzymes and therefore would accummulate in neurological lesions following nerve damage.

(4) The proposal that the "prion" agent consists only of self-replicating proteins (the "protein only hypothesis")(6) and is devoid of nucleic acids,(7) raises serious problems in molecular biology. (8)

(5) Furthermore immuno-deficient animals, such as SCID mice do not develop "scrapie" following scarification with affected brain tissue. (9) It is most unusual to find absence of immune reactivity as protective, since SCID mice readily succumb to viral and bacterial infections.

THE HYPOTHESIS THAT BSE IS A FORM OF AUTOIMMUNE DISEASE

The hypothesis is proposed that BSE is caused by crossreactive auto-antibodies evoked following exposure of cows to biological material from sheep containing bacteria which may crossreact with bovine self-antigens. Since neurological damage is the main feature of BSE, it is suggested that damage to nerve tissue occurs, probably in 2 stages: firstly the outer covering of neurones, namely the myelin sheath is damaged which exposes the nerve tissue and in the second stage neuronal damage occurs, with relative acummulation of "self-proteins" which cannot be readily hydrolysed such as "prion proteins".

Injection of brain tissue into experimental animals causes a neurological auto-immune disorder called "experimental allergic encephalomyelitis" (EAE) and a highly encephalitogenic peptide has been isolated from bovine myelin, having the following sequence: FSWGAEGQK. (10) We have used this sequence to search the Genbank and SwissProt databases for similar sequences and found 3 microbes which show partial molecular mimicry to bovine myelin: Acinetobacter calcoaceticus , Ruminococcus albus and Agrobacterium tumefaciens. (Table 1A)

Acinetobacter is a microbe found extensively in soil and water supplies, Agrobacterium is a plant pathogen causing galls, whilst Ruminococcus is found in the bowel flora of ruminants.

The sequence in Acinetobacter contains a positively charged arginine (R) and a negatively charged glutamic acid (E) thereby forming an immunogenic epitope. (Fig.1) The host protein consisting of arginine-phenyl alanine-serine and tryptophan (RFSW) may bind immunocompetent cells and antibodies against RFAW of Acinetobacter and thereby cause damage to nervous tissue. Furthermore the sequences in both Acinetobacter and Agrobacterium contain tryptophan (W), an amino acid which was found to be necessary in producing EAE, since modification of the tryptophan residue led to loss of encephalitogenic activity. (10)

We have also used the bovine "prion sequence" NMKHVAG (11), to search the databases for similar sequences in microbes which may show partial molecular mimicry. Two sequences were found, both in the same microbe:

NMKHVAG	Bovine "prion"
NMKQMSG	Escherichia coli colicin M (Table 1B)
QMKNMGG	Escherichia coli signal recognition protein (Fig.2)

If BSE is an auto-immune disease, then elevated antibody levels to the bacteria showing molecular mimicry should be present during active phases, when acute phase reactants such as serum C-reactive protein levels are elevated. The pathological mechanism could be similar to rheumatic fever, rheumatoid arthritis (12) or ankylosing spondylitis (13) where crossreactive epitopes have been described in bacteria (site of infection), such as Streptococcus pyogenes (tonsillitis), Proteus mirabilis (cystitis) and Klebsiella pneumoniae (ileal Crohn's like lesions) (14) respectively, which may act as autoimmune trigger factors in producing these diseases. Inadvertent feeding of cattle with supplementary foods containing meat and bone meal which could have been exposed to these common ovine (Ruminococcus and Escherichia) and environmental (Acetinobacter and Agrobacterium) bacteria may have evoked immune responses with autoimmune activity.

The 2 theories have different economic implications: the prion-virus hypothesis proposes that cows/sheep (BSE/scrapie) are infected by the rion/virus agent and therefore such animals should be culled with attendant financial costs. The autoimmune hypothesis proposes that neuronal damage is caused by immune processes similar to EAE, following exposure in the gut to bowel bacteria carrying sequences resembling myelin and nervous tissues. Since the tissue damage is caused by self proteins, namely autoantibodies, the affected animals are not "infected" and treatment is to remove the offending crossreactive antigenic bacteria from the bowel flora.

Another important feature of BSE has been the demonstration that maternal transmission has occurred from dam to calf, but a similar situation is well described in human pathology, where pregnant women suffering from myasthenia gravis or thyrotoxicosis can transmit the disease via transplacental transfer of maternal IgG to their offsprings. After birth, the neonates progressively recover from the disease as maternal IgG autoantibodies subside over time.

The observation that some children who received human growth hormone contaminated by brain tissue, subsequently developed a CJD like disease is similar to the situation of experimental animals developing EAE following injection of bovine myelin.

The autoimmune hypothesis predicts that BSE affected animals should have elevated levels of antibodies to whole bacteria carrying crossreacting self-antigens, as well as to short peptides containing such sequences (bovine myelin, host encoded prion proteins) and these could be helpful in establishing an early diagnosis.

. . .

Acknowledgements: The authors would like to thank the Trustees of the Middlesex hospital for their support.

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TABLE

Comparison of amino acids of bovine myelin (A) and prion proteins (B) to microorganisms from Genbank and SwissProt which have similar sequences in other proteins.

Source	Amino acids	Positions	Locations
Part A: Bovine myelin comparisons			
Bovine myelin	LSRFSWGAE	110 - 118	
Acinetobacter calcoaceticus	ISRFAWGEV	41 - 49	4-carboxy-muconolactone decarboxylase
Agrobacter tumefaciens	YTRFTWGAP	693 - 701	Beta-glucosidase
Ruminococcus albus	YTQFEISAE	274 - 282	Beta-glucosidase
Part B: Prion proteins comparisons			
Bovine prion	NMKHVAG	119 - 125	
Human prion	NMKHMAG	108 - 114	
Escherichia coli	QMKQMSG	340 - 346	E.coli signal recognition protein
Escherichia coli	NMKQMSG	118 - 124	E.coli colicin M

Alphabetical letters refer to biochemical symbols for amino acids.

FIGURE 1.

Comparison of space filling models, using Alchemy III (Tripos ASSOC Inc, St.Louis, USA) of A.calcoaceticus, bovine myelin and A.tumefaciens. (Black = carbon, red = oxygen, blue = nitrogen, yellow = sulphur)

FIGURE 2.

Comparison of space filling models of E.coli signal recognition protein, bovine prion and human prion.

DIAGNOSIS and PREVENTION of SPONGIFORM DISEASES

Field of Invention

Understanding of the process of spongiform diseases

Diagnostic tests for the detection of antibodies to various micro-organisms responsible for spongiform diseases

Vaccines against these micro-organisms.

Background to Invention

It has been suggested that Bovine Spongiform Encephalopathy (BSE) and similar spongiform diseases are caused by infection with agents known as prions, and this is at present the generally received model.

The prions are considered to be independent infectious agents, which are extremely difficult to determine by means of assay, and equally difficult to de-activate, hence the lack so far of any cure for BSE.

The current method of determining BSE is by *post mortem* pathological examination.

The existence of the prions is not in question, but it has not been demonstrated that they are free exogenous infectious agents, and much of the existing experimental data is not consistent with their being so.

The Problem the Invention Overcomes

There has recently been a major increase in the incidence of BSE in European. and in particular British, beef and milk herds. This has led to a major lack of confidence in the safety of beef from these herds, because it is believed that BSE is transmitted by prions which are believed to be independent transmissible infectious agents, and which are difficult or impossible to inactivate, and it is believed that these prions are capable of transmission between species

As a result of this the sale of beef products has dropped drastically, causing enormous financial losses to farmers individually, and losses to the national economy in the decrease of exports

Tens of thousands of cattle are presently being culled in the hope of eliminating (or accelerating the elimination of) BSE, and thus aggravating the economic losses by loss of stock, and the requirement on the governments to pay massive compensation for stock culled.

Description of the invention

We have now demonstrated an alternative model for the cause of BSE, which more nearly fits the observed facts concerning the disease and its transmission. This model is based on the auto-immune process, and recognizes that the prion is not an infectious agent passing between hosts, but a degraded form of normal endogenous brain protein resulting from attack by the victim's own immune system. This attack is by auto-antibodies which have been produced as a result of exposure to specific bacteria which carry antigens which mimic sequences present in normal brain proteins, such as prions and bovine myelin. The new model is equally applicable in relation to CJD and scrapie in humans and sheep respectively. According to this model, BSE is not a transmissible disease, either within species or between species. This new model is the primary invention

Practical realisations of the invention

Based on this model, we have produced a diagnostic assay to determine whether a living cow is or is not suffering from BSE, and vaccines to protect cows against contracting BSE. The target bacteria in these products are Acetinobacter calcoaceticus, Agrobacterium tumefaciens, Ruminococcus albus, and Escherica coli, all of which have been demonstrated to contain sequences which mimic parts of brain proteins.

Similarly, assays and vaccines relating to CJD and scrapie can be produced. These assays and vaccines form part of the invention.

The invention also includes any assays and vaccines or therapeutics which are used in the diagnosis, prevention or treatment of spongiform disease in any species and are based on the new model of this invention.

The invention also includes such assays and vaccines or therapeutics which are used in the diagnosis, prevention or treatment of any other auto-immune diseases which conforms to the new model of the present invention , where such assays and vaccines or therapeutics can be produced to counter those bacteria which mimic the relevant endogenous protein.

Benefits

An assay to diagnose early cases of BSE would have the complementary benefit of demonstrating that a given animal was not suffering from BSE, and need not therefore be culled. Culling could therefore be on a case by case and rational basis rather than whole herds at a time to achieve "over-kill".

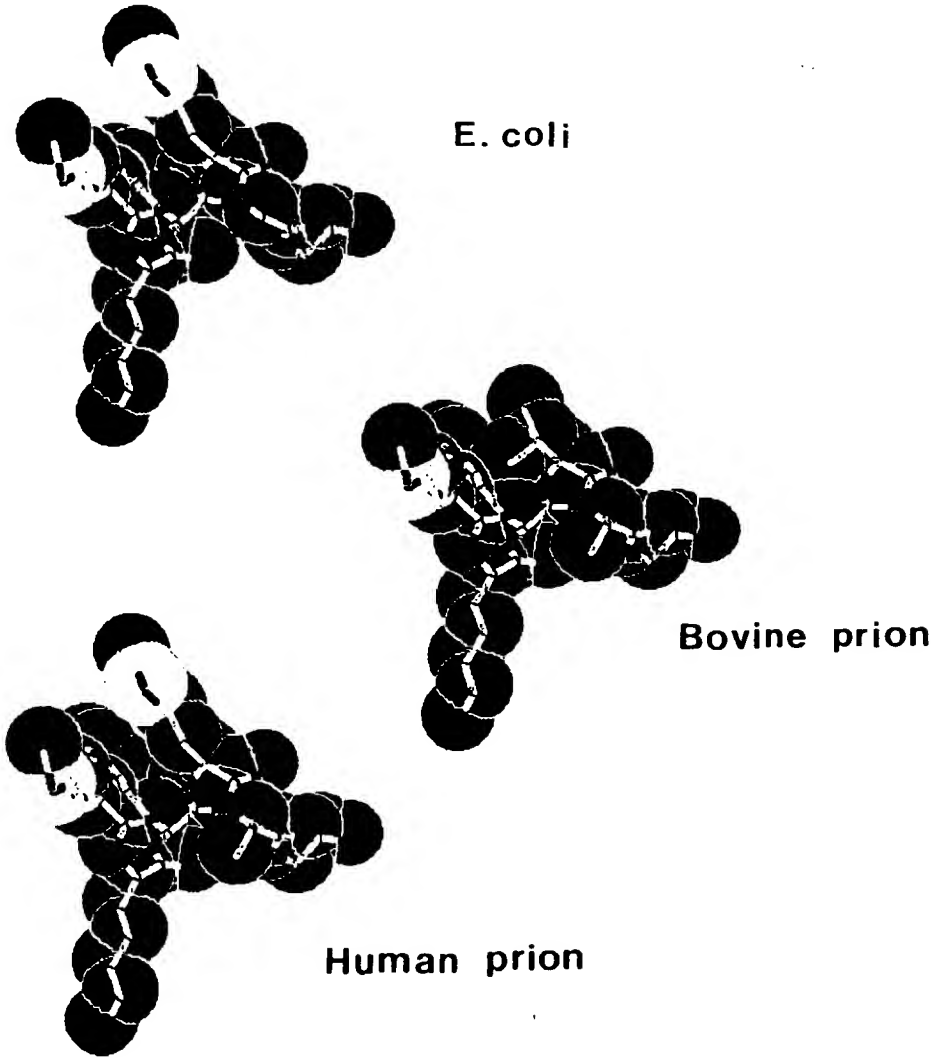
Better still, the new model of the disease indicates that it is not transmissible, and therefore an animal which is suffering from BSE is not a risk to others, and the only need to destroy it is on humane grounds.

The net effect of this reduction or elimination of culling would be a major economic benefit to farmers and to the nations of Europe.

Clearly the wider applications of the invention would lead to major advances in the prevention and treatment of a variety of diseases.

Further Supporting Information

Two papers describing the scientific basis of the invention in more detail are attached, one intended for publication in the Lancet, and one seeking funding from the Ministry of Agriculture Farming and Fishery. These papers form part of this application.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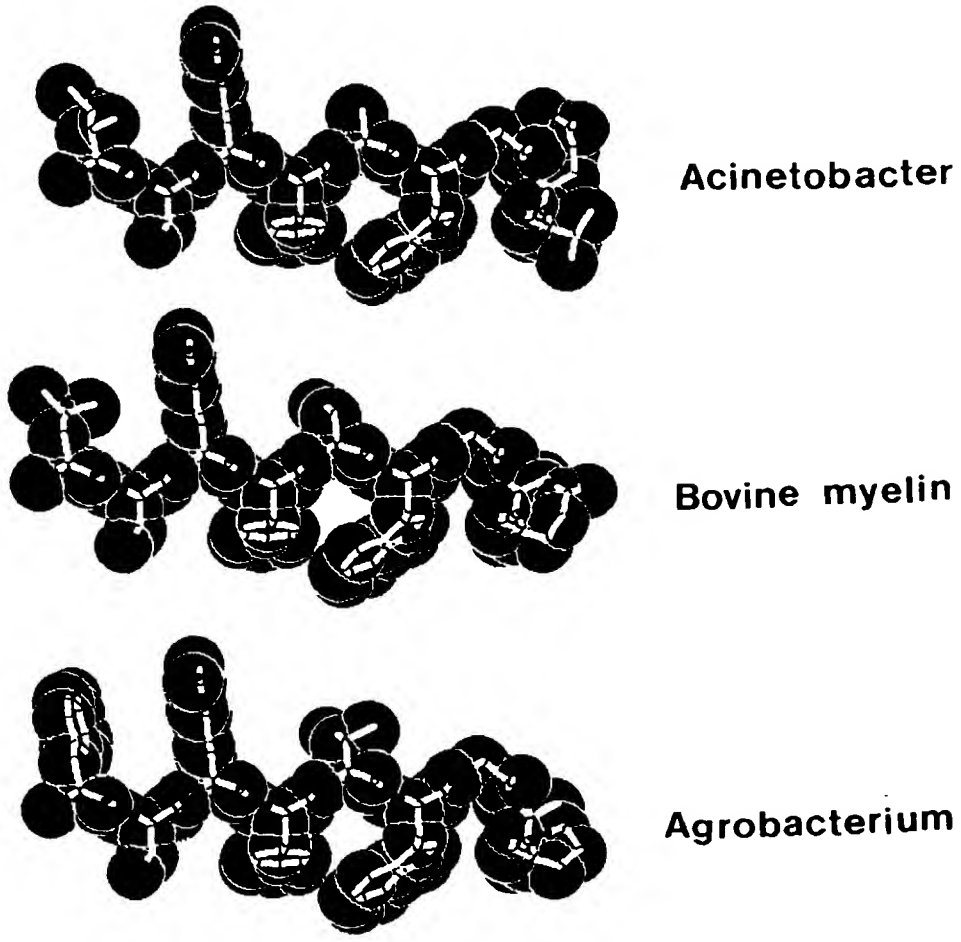
Bovine prion
Human prion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